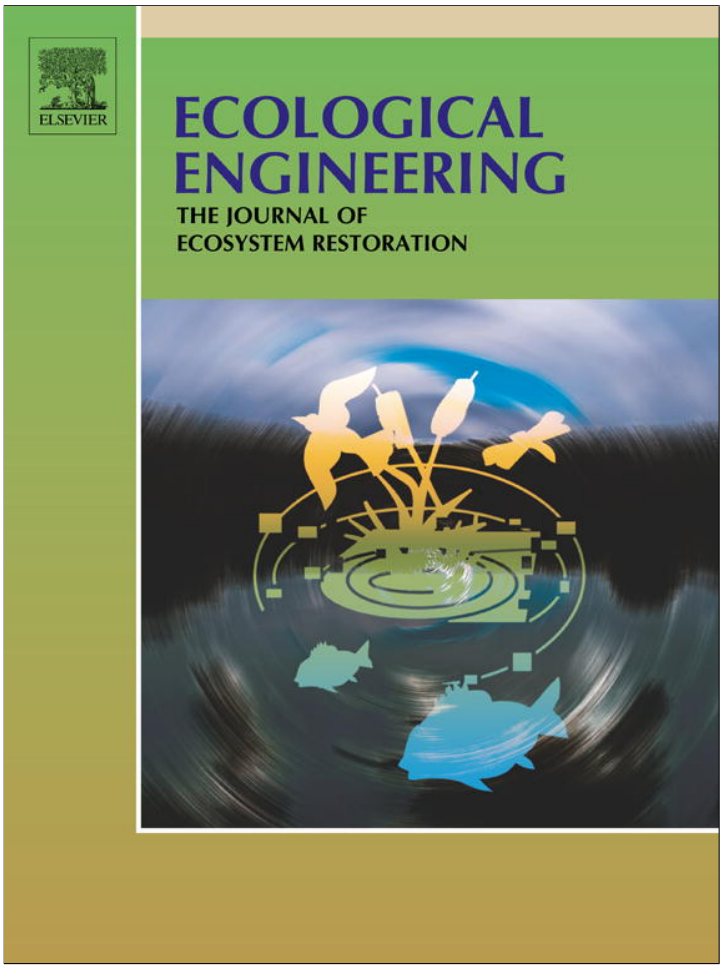

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Research paper

Effect of pH control by means of flue gas addition on three different photo-bioreactors treating urban wastewater in long-term operation

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ABSTRACT

Effect of pH control with flue gas has been studied in two high rate algal ponds (HRAPs), one with a carbonation sump station (HRAP + S), and a tubular airlift photobioreactor (TPBR) treating urban wastewater. Flue gas, from 1600 MW combined cycle plant, addition (4–5% volume CO_2) not only increased biomass productivity but also improved efficiency of total nitrogen removal (TNRE) and total phosphorus removal (TPRE). The differences between the HRAP and HRAP + S were significant at all the flue gas injection flow rates tested. HRAP + S reached maximum TNRE, TPRE and biomass productivity ($92.15 \pm 1.45\%$, $95.10 \pm 0.84\%$ and $19.77 \pm 0.38\ g\ m^{-2}\ d^{-1}$, respectively) at a flow rate of $15\ L\ min^{-1}$, while the HRAP reached similar productivity levels at $20\ L\ min^{-1}$. TPBR showed an initial lower carbon limitation than HRAP and HRAP + S, but nevertheless a strong inhibition was observed in TPBR at the end of the test. Flue gas addition promotes the production of biomass with less nitrogen reserves and consequently with higher lipid content because of the nutrient limitation stress.

1. Introduction

The use of microalgae for nitrogen and phosphorus removal in urban wastewater treatment plants (WWTPs) has been widely investigated and demonstrated (Mata et al., 2010; Ruiz et al., 2011; Arbib et al., 2012). One of the most serious drawbacks of microalgae cultivation in treated urban wastewater is the low C:N:P ratio of the wastewater obtained, about 20:8:1 (Woertz et al., 2009). That ratio contrasts markedly with the theoretical values proposed by Redfield (1958) for the balanced growth of an algal population of 106:16:1. One must conclude, accordingly, that typical urban wastewater contains insufficient carbon to fully support the optimum algal production. This carbon deficiency contributes to a slow rate of growth and, consequently, to an incomplete nutrient removal (Woertz et al., 2009; Park et al., 2011). Therefore the addition of an external carbon source (e.g. CO_2) to algal cultures has been shown to increase significatively the biomass productivity (Azov et al., 1982; Lundquist et al., 2009; de Godos et al., 2010). Woertz et al. (2009) reported an enhancement of biomass concentration from 317 to $812\ mg\ L^{-1}$ when CO_2 was added to a semi-continuous culture of a microalgae consortium in a preliminary clarified wastewater. The addition of CO_2 to microalgae culturing systems not only improves the carbon availability for algal growth and the nutrient removal, but also serves to mitigate pH inhibitions related to the higher free ammonia concentrations at high pH (Heubeck et al., 2007). On this point, it is widely reported that at a pH above or below 8 can produce a significant decrease not only in biomass productivity but also in nutrient removal efficiency, at pH above 9 took place the ammonia volatilization and phosphorus precipitation (Woertz et al., 2009; Kong et al., 2010; Park et al., 2011).

Currently, two main systems for microalgae cultures can be distinguished: one open and the other closed. Each has its own characteristics regarding the operation and control of the process. Regarding the biomass productivity, open pond systems are less efficient when compared to closed photobioreactors, and this can be attributed to several factors such as light limitation, inefficient mixing, and deficiency of CO_2 mass transfer (Chisti, 2007).

Focusing on the CO_2 mass transfer, the delivery and utilization of CO_2 in an algae mass production system are significant factors for the design and operation of the cultivation system. It is reported that, in closed systems, the CO_2 mass transfer is higher than in

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Table 1
Mean average nutrient composition of the influent wastewater during the four different periods of continuous mode of operation. Total nitrogen (TN, mg N L^{-1}); total phosphorus (TP, mg P L^{-1}); soluble chemical oxygen demand (COD, mg O_2 L^{-1}).

Nutrient	Period I (no flue gas)	Period II (10 L min^{-1})	Period III (15 L min^{-1})	Period IV (20 L min^{-1})
TP (mg P L^{-1})	2.18 ± 0.15	2.07 ± 0.16	2.25 ± 0.14	1.98 ± 0.11
TN (mg N L^{-1})	26.93 ± 1.88	24.65 ± 1.23	25.35 ± 1.65	22.86 ± 2.43
SCOD (mg O_2 L^{-1})	78.00 ± 3.00	81.00 ± 3.79	81.33 ± 2.58	78.88 ± 6.58

open systems, due mainly to the longer contact time between the gaseous phase (CO_2) and the liquid phase (culture media) (Pires et al., 2012). Open systems are shallow reactors (30 cm depth), with a very large surface-to-volume ratio. In most cases CO_2 is supplied in the form of fine bubbles from the bottom of the pond (from 30 cm depth), and therefore the residence time of the bubble is not sufficient to allow all the CO_2 injected to dissolve in the culture medium (Mata et al., 2010). Consequently, some of the CO_2 can easily be lost to the atmosphere and may result in reduced biomass productivity (Brennan and Owende, 2010). Oswald (1988) proposed an effective method for transferring CO_2 in open systems, which consists of a sump counter-current carbonation station; Sheehan et al. (1998) reported that the carbonation system was essentially 100% efficient in CO_2 transfer, and the overall CO_2 utilization was higher than 90%.

The aim of the study described here was to investigate the effect of the addition of real flue gas from 1600 MW combined cycled plant of Iberdrola (Arcos de la Frontera, Spain) (4–5% CO_2) when the algae *Scenedesmsus obliquus* is cultivated simultaneously in the effluent of a wastewater treatment plant, in three different photobioreactors (PBRs): (1) a conventional high-rate algal pond (HRAP); (2) a high-rate algal pond with carbonation sump station (HRAP + S); and (3) a closed tubular airlift photobioreactor (TPBR). Biomass growth rate, nutrient removal capacity and biochemical composition were evaluated in the three PBRs.

2. Materials and methods

2.1. Microorganism

The microalga strain used in this study was *S. obliquus* (SAG 276-10) (*S. obliquus*), obtained from the Algae Culture Collection (SAG), Göttingen University (Germany). The inoculum for the experiments was cultivated in non sterilized treated urban wastewater at 20 ± 1 °C and 250 μmol cm^{-2} s^{-1} light intensity under a 14:10 light:dark cycle, the inoculum was checked by microscope periodically and the dominance species was *S. obliquus* during the entire period of experimentation.

2.2. Culture media

The feedstock used was pre-treated wastewater (primary and secondary) from the wastewater treatment plant (WWTP) located in Arcos de la Frontera (36°44′56.56″ N, 5°47′37.12″, Southern Spain). The wastewater was collected after the preliminary screening, primary sedimentation, activated sludge and secondary sedimentation processes. The experiments were carried out firstly in batch mode, and after that in four different continuous flow periods. Table 1 shows the average nutrient composition of the influent wastewater to the PBRs during the four continuous flow periods.

2.3. Photobioreactors

The experimental designs consist in three different photobioreactors (PBRs), two open systems and a closed system (Fig. 1A–C).

The open systems consisted of a typical raceway pond, also called high rate algal pond (referred to henceforth as HRAP and HRAP + S). Both were constructed in fiberglass; their water surface area was 1.98 m^2 and their dimensions were: length 2525 mm; width 770 mm; and depth 450 mm. The main difference between the two open systems was that the HRAP + S was constructed with a carbonation sump station (width 300 mm, depth 1000 mm), with an internal vertical baffle divider and spanning the full width of the channel, opposite to the mixing station (Fig. 1B), in HRAP + S flue gas was added in counter-current flow, through a tube diffuser. The culture in the HRAP and HRAP + S was mixed mechanically with a motor-driven paddle wheel with 4 blades, at 5 rpm, reaching a flow velocity of between 20 and 30 cm s^{-1}. The paddle wheel sits in a depression on the pond bottom; the depression serves to reduce the back-flow effect (Dodd, 1986). The final working volumes were 533 and 593 liters (L) for the HRAP and HRAP + S, respectively.

The closed system consisted of a tubular airlift photobioreactor (TPBR), designed and constructed according to the recommendations of Pirt et al. (1983) and Molina-Grima et al. (2001). The TPBR was divided in two parts: the first is a solar receiver, which consists of 12 straight transparent polymethyl methacrylate (PMMA) tubes, each with a length of 2000 mm and external diameter of 110 mm. The straight tubes are joined into a vertical loop configuration, also called a 'fence-type' configuration. The working volume of the solar loop receiver was 330 L; the second part is an airlift-driven system (ADS) used to re-circulate the culture through the solar receiver at low shear stress and to strip the excess of oxygen from the culture media. The ADS (Fig. 1C) was formed by three main sections: riser, downcomer and degasser. The riser was connected vertically to the end of the loop of the solar receiver by a 90° PVC bend. As the riser is also constructed with a 110 mm dia. PMMA tube, it can be considered part of the solar receiver, giving a total volume for the solar receiver of 350 L. Air was injected at the bottom of the riser, and so the bubbling air promotes culture mixing, degassing and circulation through the solar receiver. The downcomer was joined vertically by a 90° PVC bend to the beginning of the solar receiver. The gas–liquid separator (degasser) was connected to the riser and the downcomer, with the bottom of the degasser inclined to avoid the sedimentation of the solids (Molina-Grima et al., 2001). The total height of the ADS was 3000 mm. The total working volume of the dark zone (degasser + downcomer) was 30 L, which, in accordance to the recommendations of Molina-Grima et al. (2001), was less than 12% of the total volume. The flue gas injection in TPBR was made at the bottom of the downcommer part of the airlift system. The flue gas addition occurs when the pH of the culture increases above 8. When this happens, an electro-valve was actuated by the action of a controller and flue gas was released into each PBR.

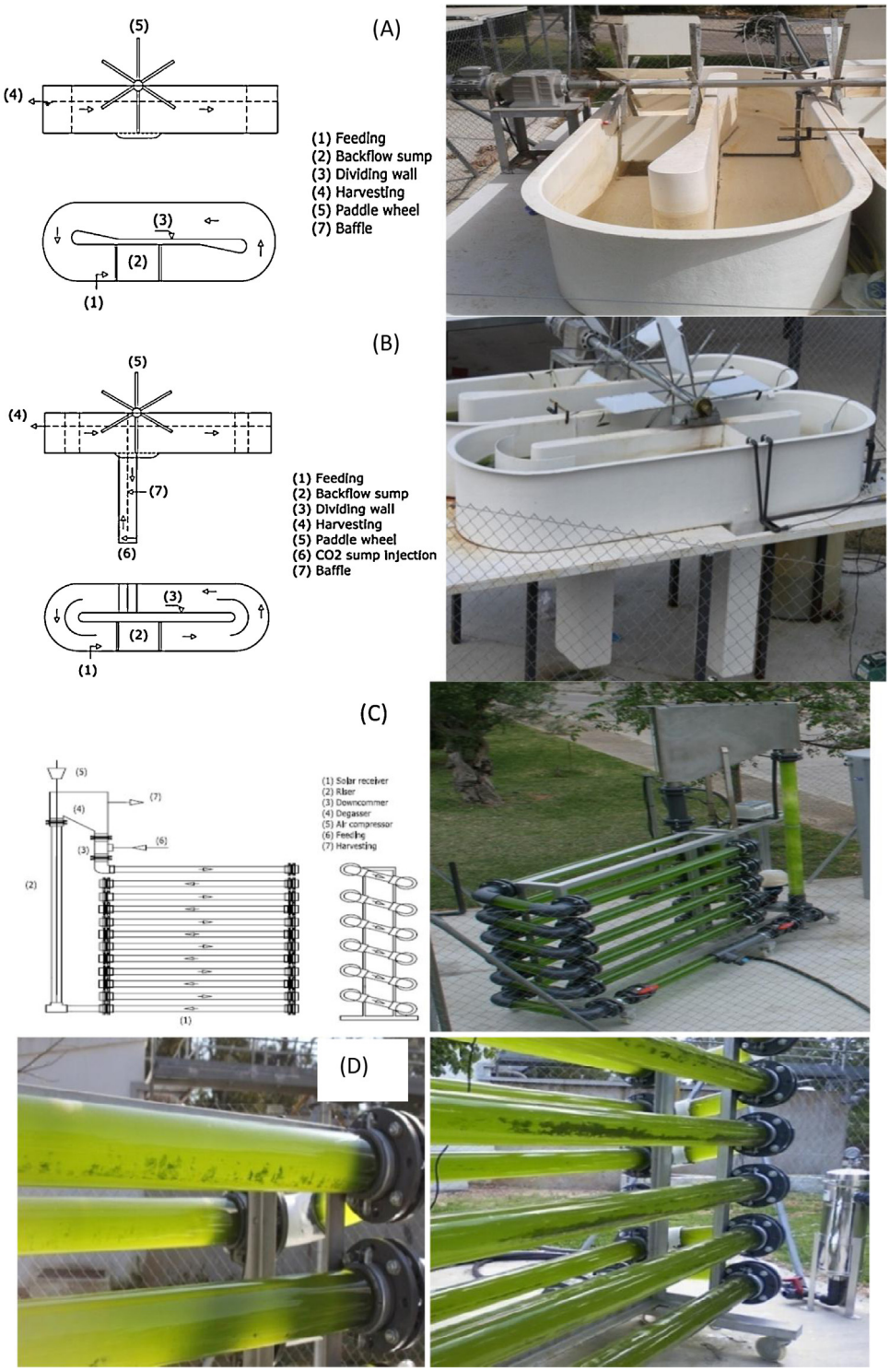

Fig. 1. (A) Flow diagram (left) and real high rate algal pond (HRAP) (right; (B) flow diagram (left) and real high rate algal pond with carbonation sump station (HRAP+S) (right); (C) flow diagram (left) and real airlift tubular photobioreactor (TPBR) (right); (D) biofouling in TPBR. Left picture: initial appearance of biofouling (day 32); right picture: biofouling at the end of the experiment (day 62).

2.4. Experimental design

The PBRs were installed and operated from April 3rd, 2012 to June 4th 2012 (62 days) in the WWTP located in Arcos de la Frontera (Province of Cadiz, Spain).

The PBRs were filled with wastewater and inoculated with *S. obliquus* (inoculums accounted for 10% of the total working volume of each reactor). They were operated firstly in batch mode (BM), with the aim of obtaining the most appropriate dilution rate or hydraulic retention time (HRT) to be applied: the HRT should be long enough to prevent the wash-out effect in the treatment stage but not too long, because of the nutrient limitation associated with a long HRT. The procedure was similar in all the pilot plants: operating in BM until the stationary phase was reached (when the increase

of biomass was less than 1% per day for 3 consecutive days). After that, feeding pumps were connected at a pre-determined flow rate to achieve the appropriate HRT, this was 4 days for TPBR and 8 days for HRAP and HRAP + S.

Once the steady state was reached, the PBRs were operated firstly without addition of flue gas (4–5% CO_2) (period I), and then three different flow rates were tested: 10, 15 and 20 L min^{-1} which represents periods II, III and IV, the flue gas flow was continuous in each period, the controlling variable was the pH and the manipulated variable was the flue gas flow.

2.5. Analytical methods

Liquid samples for nutrient consumption analysis were extracted on three days each week and then filtered through a fiber filter of 1 μm pore diameter (PALL Corporation, Type A/E) to separate biomass. Total nitrogen (TN) and total phosphorus (TP) were determined based on the method proposed by Köthe and Bitsch (1992): 10 mL of the sample and 1.5 micro-spoons of OXISOLV® (Merck KGaA, Darmstadt, Germany) were mixed, then incubated at 100 °C for 60 min and cooled to room temperature. Next, nitrate determinations were performed according to the Spectroquant® test kit (Cod. 1.14773.0001 (Merck)), and phosphates were determined according to the 4500-P E method (APHA, AWWA, WEF, 1992). Soluble chemical oxygen demand (SCOD) was determined according to Standard Methods 5220-D (APHA, AWWA, WEF, 1992).

Microalgae biomass concentration was measured daily by optical density at 680 nm (OD_{680}). When necessary, samples were diluted appropriately to ensure values in the range of 0.1–1.0. In order to convert OD_{680} values to biomass as dry weight, a calibration curve was developed (SS (mg L^{-1}) = 0.9590·(OD_{680}) − 0.075; r^2 = 0.991). Biomass dry weight as suspended solids was determined gravimetrically according to Standard Methods (APHA-AWWA-WPCF, 1992).

During the four periods of the continuous mode operation, the biomass composition in all the pilot plants was analyzed. Biomass was harvested by centrifugation (Centrifuge Mixtasel-BL Selecta®) at 4200 rpm for 10 min. The resulting pellets were rinsed twice with de-ionized water, for re-suspending and centrifuging. Algae pellets were dried in a lyophilizer (Labconco, FreeZone Triad Cascade Benchtop). The elementary analysis (percentage of C, N, H and S) of the biomass was performed by a Leco® CHNS 932 analyzer. Biomass lipid concentration was determined in duplicate. Lipids were extracted according to a modified method reported by Wiltshire et al. (2000) and Takagi et al. (2006). To 90 mg of lyophilized pellets, 12 mL of 2:1 trichloro methane:methanol and 0.6 g of analytical grade quartz were added, and the mixture was sonicated in a bath (60 kHz; 360 W) for 90 min. Extraction was done twice and the two extracts were mixed, centrifuged and filtered to ensure quartz separation. The filtrate was evaporated under reduced pressure in a rotary evaporator. The remainder was dried at 100–105 °C for 12 h and weighed as total lipids.

3. Results and discussion

3.1. Effect of flue gas on biomass growth rate

As temperature and light availability play important roles in the outdoor cultivation of microalgae, ambient temperature and the temperature inside each PBR were monitored (Fig. 2A), as well as the maximum and average daily external solar irradiance (μmole m^{-2} s^{-1}), as shown in Fig. 2B.

Fig. 2C and D shows the evolution of biomass in the three PRBs. These plants were operated firstly in batch mode (BM) without flue gas addition. BM operation lasted 9 days for the TPBR (Fig. 2D) and 11 days for HRAP and HRAP + S (Fig. 2C). The final concentration achieved in the TPBR was 2 and 2.25 times higher than in the HRAP and HRAP + S, respectively. Therefore, under the same conditions of operation and the same culture medium, the growth rate in BM in the TPBR was higher than in the HRAP and HRAP + S. This behavior could be due to the greater light limiting effect in the open systems (larger light path, 300 mm) in comparison with the TPBR (light path of 110 mm).

3.1.1. Period I

Once the steady state was reached, the reactors were operated in continuous mode with a hydraulic retention time (HRT) of 4 days for the TPBR, and 8 days for HRAP and HRAP + S. In the first period of continuous mode (designated period I), which comprised from day 10 to day 14 in the TPBR, and from day 12 to day 16 for the HRAP and HRAP + S, no flue gas was added for the subsequent evaluation of the influence of the gas sparging. The average productivity by area (g SS m^2 d^{-1}) achieved during this period was also higher in the TPBR than in the HRAP and HRAP + S reactors (10.17 ± 0.12, 10.23 ± 0.11 and 28.50 ± 0.40 g SS m^{-2} d^{-1} for the HRAP, HRAP + S and TPBR, respectively) (Fig. 2E). During period I, the biomass concentration remained almost constant in the three photobioreactors as a consequence of the stability of the climatic conditions (Fig. 2C and D) and the nutrient composition of the influent (Table 1).

3.1.2. Period II

After operating without CO_2 addition, flue gas was injected into each of the PRBs at three different flow rates, 10, 15 and 20 L min^{-1} (designated periods II, III and IV). In Fig. 3A–C the different pH profiles obtained over the course of 24 h for each PBR at a flue gas flow rate of 10 L min^{-1} can be appreciated. A similar qualitative pH behavior was observed in the subsequent trial periods (data not presented).

The most significant difference observed between the PRBs was during the night-time; according to the respiration theory, during the night, i.e. the dark phase of photosynthesis, the respiration releases CO_2 which produces a decrease in the pH. This pH reduction could be clearly observed in the HRAP and HRAP + S (Fig. 3B and C); the average pH values recorded during the night-time were 7.72 ± 0.09 and 7.67 ± 0.08 for the HRAP and HRAP + S, respectively.

In the TPBR, the pH pattern is different: a slight increase in pH during the night can be observed (Fig. 3A), even reaching the set pH value of 8.0 and activating the CO_2 control system at about 03.00 h. This increase in pH could be explained by the striping of CO_2 generated by respiration by microalgae during the night period by the air injected in the airlift pump system. Similar behavior was previously reported by Rebolloso-Fuentes et al. (1999) and Valdés et al. (2012) when they operated a tubular airlift photobioreactor and a bubble column, respectively.

The intervals of flue gas injections were closely related to the photosynthetic activity; therefore the daily external solar irradiance (ESI) directly influenced the pH of the culture. In Fig. 3D, the variation of the ESI during a 48 h period is illustrated; ESI presents a similar Gaussian-like variation during the two-day period, reaching 0 μmole m^{-2} s^{-1} in the night-time (from 21.00 PM to 8.00 AM) and the maximum, up to 2000 μmole m^{-2} s^{-1}, between 13.00 and 17.00 PM. In the first hours of the daylight period, the pH started to increase in all the PRBs (Fig. 3A–C), and the increase was greater in the TPBR than in the HRAP and HRAP + S, due to the higher photosynthetic activity in the TPBR associated with the smaller light path (110 mm).

As can be observed in Fig. 3A, the first injection of flue gas in the TPBR took place at 7.00 AM, while in the HRAP and HRAP + S it took place later, at 10.00 AM. Once the ESI increased, the interval

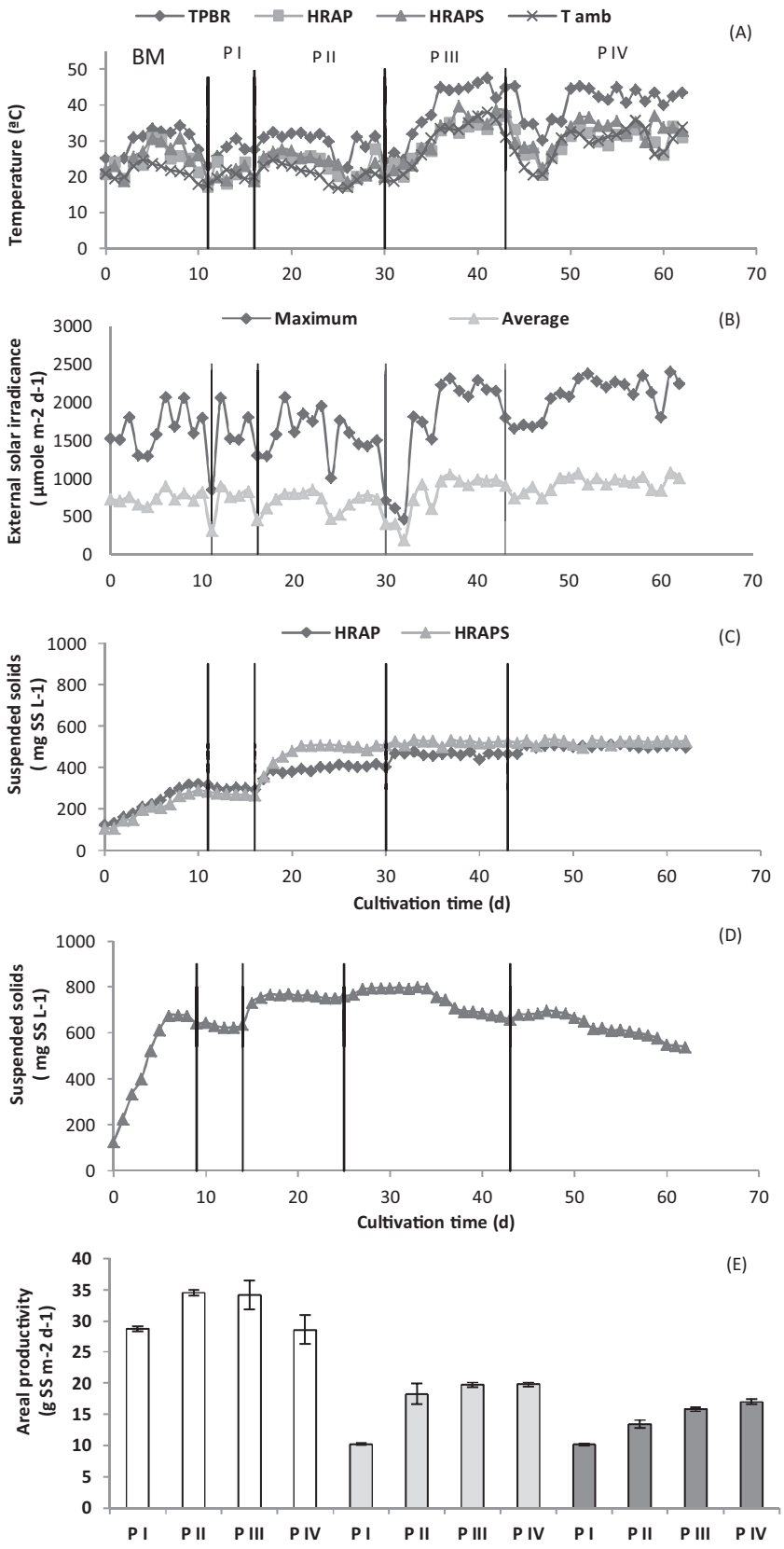

Fig. 2. (A) Time course of temperature evolution (°C); (B) maximum and average daily external solar irradiance (μmole m^{-2} s^{-1}); (C) time course of biomass concentration as suspended solids (mg SS L^{-1}) in the high rate algal pond (HRAP) and high rate algal pond with CO_2 sump (HRAP + S); (D) time course of biomass concentration as suspended solids (mg SS L^{-1}) in the airlift tubular photobioreactor (TPBR); (E) biomass productivity by area (g SS m^{-2} d^{-1}). Key: white bars (TPBR); gray bars (HRAP + S); and dark bars (HRAP) in the four different periods of continuous mode of operation.

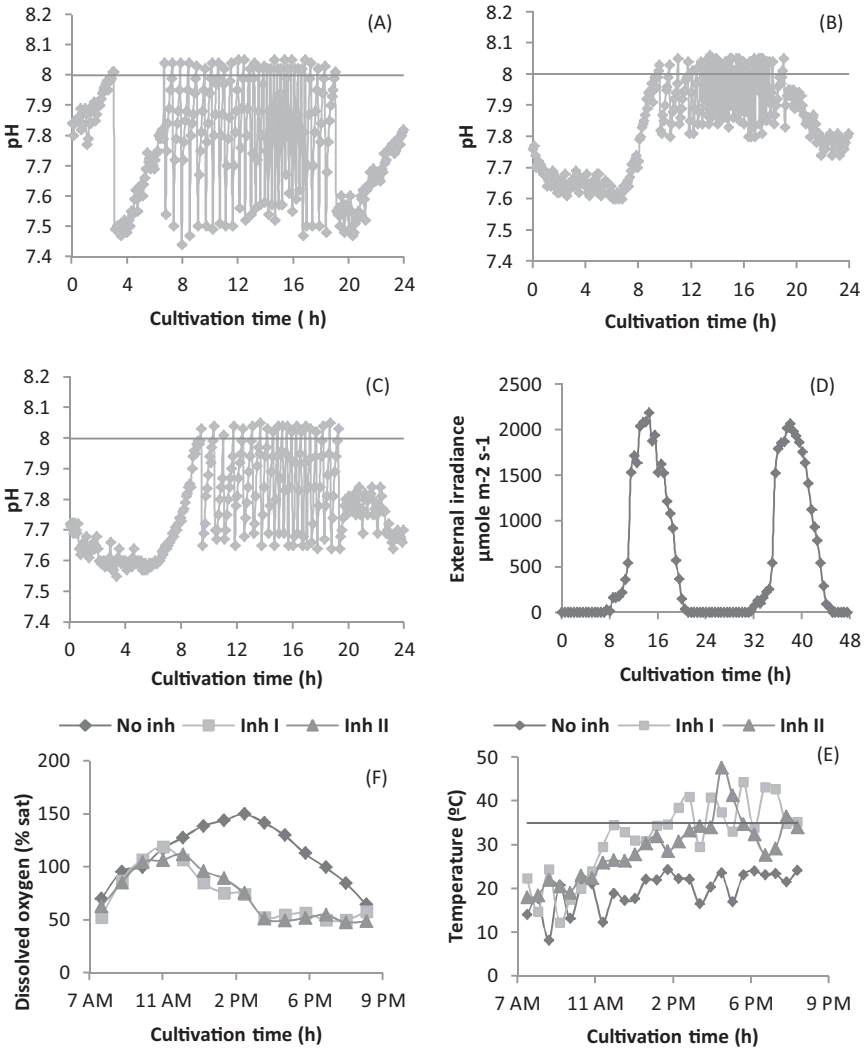

Fig. 3. (A–C) Profile of pH values during a 24 h period, in the tubular airlift photobioreactor (TPBR), the high rate algal pond (HRAP) and the high rate algal pond with carbonation sump station (HRAPS), respectively; (D) daily external solar irradiance during a 48 h period (for all plants); (E) dissolved oxygen concentration (% sat); and (F) temperature (°C) in the tubular airlift photobioreactor (TPBR) during the daytime, with no inhibition (no Inh) and with inhibition (Inh I and Inh II), respectively (average values recorded for three separate days).

between CO_2 injections became shorter in all the PRBs (Fig. 3A–C); during the hours following mid-day (from 13.00 PM to17.00 PM), the maximum number of CO_2 injections triggered per hour was 7 in the HRAP but less in the others: 3 in the HRAP + S and 4 in the TPBR.

During period II, in all PRBs, a significant increase of biomass was observed in comparison with the preceding period I. The mean average ambient temperature and external solar irradiance values (Fig. 2A and B) as well as the nutrient influent concentration (Table 1) during period II showed no significant differences with respect to the preceding period I. Therefore, the increased biomass obtained can be attributed solely to the effect of the addition of flue gas. Woertz et al. (2009) and Faria et al. (2012) also reported a significant increase in the microalgae biomass (*Chlorella* sp.) associated with the addition of dissolved carbon to the culture medium.

In this study, the lowest increase (15.89%) was observed in the TPBR; this was probably due to the lowest carbon limitation, compared to the HRAP and HRAP + S, associated with the CO_2 added in the air stream of the airlift pump system. As commented previously, the mass of CO_2 injected in the HRAP was higher than in the HRAP + S in hours following mid-day,and it was expected to achieve higher biomass concentration in HRAP, however HRAP + S suffered a biomass increment 2 fold higher than HRAP. Therefore, this difference indicate that the CO_2 added in HRAP + S was more efficiently assimilated than in HRAP therefore the very high amount of CO_2 injected per hour recorded in the HRAP was due to a lower CO_2 transfer and not to a higher photosynthetic activity, it seems that the CO_2 added in the HRAP + S was more efficiently assimilated than in the HRAP, which demonstrates the benefits of the CO_2 sump of the HRAP + S.

3.1.3. Period III

Once the concentration remained constant at a flue gas flow rate of 10 L min^{-1}, the flue gas rate was increased to 15 L min^{-1}, for period III. During this period III, the mean average ambient temperature increased significantly with respect to period II, with the average values for period II and III being 20.7 ± 2.5 and 30.4 ± 6.4 °C, respectively (Fig. 2A); however, the ESI values showed no significant differences between these periods (Fig. 2B). The increase of biomass between periods II and III was lower than between periods I and II, which indicates that the injection of CO_2 at a flow of 10 L min^{-1} was high enough to surpass the majority of the

carbon limitation occuring in TPBR without CO_2 addition. The TPBR showed an initial increase of 4.29% but, as the trial proceeded, around day 34, the biomass started to decrease sharply (Fig. 2D). The main reason for this sharp decrease was the extremely high temperatures reached in the TPBR. As can be observed in Fig. 2A, the temperature in the TPBR during the entire operation in CM was almost 10 °C higher than the ambient temperature. When the temperature inside the TPBR started to exceed 35 °C (by day 36 of the experiment), microalgae began to adhere to the surface of reactor, and after 8 days, the biofouling was severe (Fig. 1D). A similar increase in biofouling has previously been reported at extremely low temperatures (Arbib et al., 2013). Pulz (2001) reported that, for many algae, the optimal temperature under conditions of maximum algae growth varies between 28 and 35 °C; above or below this temperature biomass productivity decreases. In order to confirm this inhibition by temperature in the TPBR, the dissolved oxygen (DO, % saturation) and temperature inside the TPBR were measured from 8.00 AM to 8.00 PM on three different days (Fig. 3E and F). When no inhibition was appreciated in the first hours of the day, the DO started at almost 65–70% saturation in air; the DO increased as the ESI increased, and reached the maximum at 3.00 PM (150% saturation in air), and from this point started to decrease as the ESI decreased. On the other hand, when inhibition took place, the DO increased in the first hours of the day, and reached the maximum DO (almost 115% saturation) between 11.00 AM and 12.00 PM, and from this point declined sharply despite the increase in the ESI. It can be appreciated in Fig. 3E that on those days when inhibition took place, from 12.00 AM the temperature was above 30 °C, and reached a maximum of 47.63 °C, whereas when there was no inhibition, the temperature was below 35 °C during the whole day.

In period III, both the HRAP and HRAP + S suffered an increase of the biomass concentration with respect to the preceding period II, by 7.66% and 14%, respectively. The increased flue gas flow affected the HRAP more than the HRAP + S, despite the biomass productivity by area in the HRAP ($15.88 \pm 03.4\ g\ m^{-2}\ d^{-1}$) still being lower than in the HRAP + S ($19.77 \pm 0.38\ g\ m^{-2}\ d^{-1}$) (Fig. 2E). These results confirm again the better transfer efficiency of the carbon dioxide sump of the HRAP + S, the effect of which is a high biomass concentration.

3.1.4. Period IV

Finally the flue gas flow rate was increased to $20\ L\ min^{-1}$ in period IV. The HRAP + S did not show any increase of the biomass with respect to that of the preceding period III; therefore the increased flue gas flow rate in the HRAP + S was unnecessary. However, the HRAP presented an increase of 8.59% in the biomass concentration compared with the preceding period. Nevertheless, in this final period of the trial, the productivity by area in the HRAP ($17.04 \pm 0.44\ g\ m^{-2}\ d^{-1}$) was still lower than in the HRAP + S ($19.81 \pm 0.35\ g\ m^{-2}\ d^{-1}$) (Fig. 2E). And it is expected that to achieve a similar rate of biomass productivity in the HRAP as in the HRAP + S, more flue gas would need to be injected; it is clear, therefore, that the HRAP + S assimilates the CO_2 injected more efficiently, and the addition of sump injection is beneficial for the CO_2 mass transfer.

3.2. Effect of flue gas addition on nutrient removal capability

Fig. 4A–C shows the evolution of COD, TN and TP in the influent wastewater and in the effluent from the PBRs during the four periods (I–IV) of operation in continuous mode.

Based on the results, the three PBRs were not able to reduce COD (Fig. 4A), the COD being higher in the effluent than in the influent wastewater of each PBR during all the periods, this fact could be related to the extracellular organic matter (EOM) released by algae

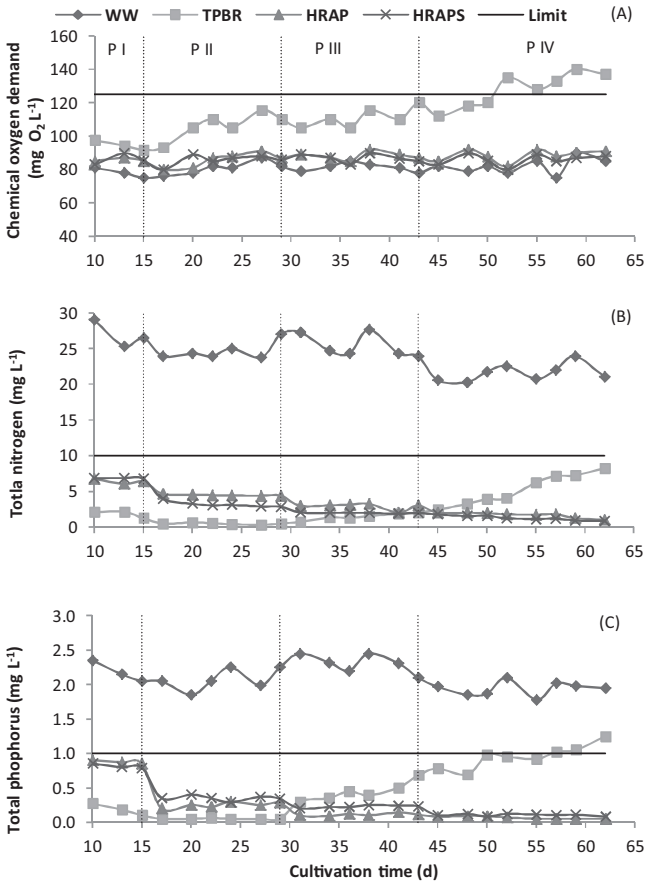

Fig. 4. Time course of nutrient content in the tubular airlift photobioreactor (TPBR), high rate algal pond (HRAP), high rate algal pond with carbonation sump station (HRAP + S) and influent wastewater (WW) during operation in continuous mode: (A) chemical oxygen demand ($mg\ O_2\ L^{-1}$); (B) total nitrogen ($mg\ TN\ L^{-1}$) and (C) total phosphorus ($mg\ TP\ L^{-1}$).

during the culture. Although it is widely reported in the literature that *Scenedesmus* sp. is capable of growing satisfactorily in photo-autotrophic, hetero-trophic and mixo-trophic modes (Zhao et al., 2012), in this case there was no mixo-trophic nor hetero-trophic growth of *S. obliquus.* Therefore the non-removal of organic carbon (COD) could be associated with the low biodegradability of the organic matter of the effluent of the WWTP (Arbib et al., 2012, 2013). COD in the TPBR showed an exponential increase during almost the entire experiment, and even at the end of the experiment, COD was above the most restrictive limit of discharge of the European Directive 98/15/CE ($125\ mg\ O_2\ L^{-1}$).

The increase in the COD was related to the severe inhibition (Fig. 2D) observed in the TPBR, because of the high temperatures, together with the lower irradiation caused by the biofouling (Fig. 1D) and to the subsequent hydrolysis of the biofouling generated. On the other hand, in both the HRAP and HRAP + S, COD concentration in the effluent was similar to that of the influent, and was always below the limit of discharge (Fig. 4B and C).

The concentrations of TN and TP in the effluents of all the PBRs, during almost all the experimental period, were below the most restrictive limits of the European Directive (98/15/CE) (Fig. 4B and C). Due to the strong inhibition observed in the TPBR (Fig. 3E and F), both TN and TP concentrations increased in the last period IV, and in the last days of operation, the TP concentration was above the limit of $1\ mg\ L^{-1}$.

Considering TN and TP removal efficiency (TPRE and TNRE), in period I there were large and significant differences between

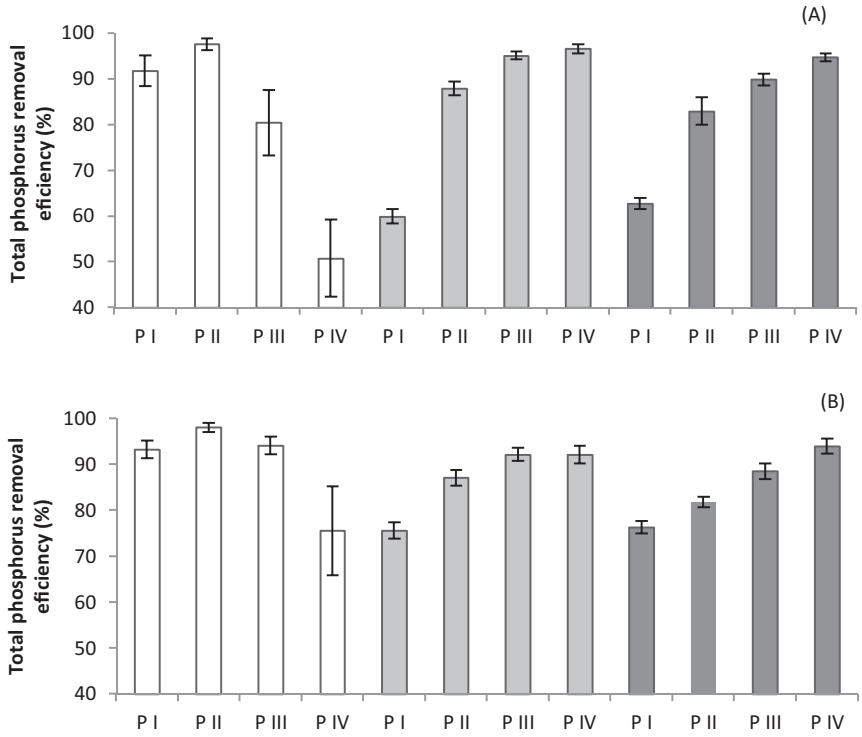

Fig. 5. (A) Total nitrogen removal efficiency (TNRE, %); (B) total phosphorus removal efficiency (TPRE, %). White bars (TPBR); gray bars (HRAP + S); and dark bars (HRAP) in the four different periods of operation in continuous mode (periods I–IV).

the closed system (TPBR) and open systems (HRAP and HRAP + S) (Fig. 5A and B). Maximum TNRE (91.75 ± 3.31%) and TPRE (93.18 ± 1.91%) were achieved in the TPBR. Since the pH in the TPBR, during period I, remained below 9 (data not presented), the main mechanism for the uptake of nutrient has been biotic assimilation (García et al., 2006). On the other hand, in the HRAP and HRAP + S, a combination of biotic and abiotic mechanisms could have been responsible for the nutrient removal. The abiotic process (ammonia volatilization and phosphorus precipitation) could have taken place because of the high pH reached (almost 10) (García et al., 2006). The average TPRE values in the HRAP + S and HRAP were 59.92 ± 1.62 and 62.69 ± 1.19%, respectively, while the average TNRE values were 75.54 ± 1.75 and 76.25 ± 1.35%, respectively. Other authors, such as Buelna et al. (1990) and Molinuevo-Salces et al. (2010), have reported a TPRE in open system between 79.10 and 93.20%, higher values than in this work.

Once the flue gas was sparged (period II), the pH was maintained at below 8 in all the PBRs (Fig. 3A–C); accordingly, all the abiotic mechanisms involving nutrient removal in the presence of flue gas should be negligible. In period II, all the PBRs presented an increase in both TNRE and TPRE (Fig. 5A and B). This improvement of the nutrient removal capability was associated mainly with an increase of biomass concentration (Fig. 2C and D). The smallest increase of both TNRE (4.92%) and TPRE (5.93%) was observed in the TPBR, together with the smallest biomass increase (15.89%); this was due to the low carbon limitation of TPBR commented previously. The maximum increase in both TPRE (31.86%) and TNRE (13.19%) was achieved in the HRAP + S, with the differences in respect of the HRAP being significant. Since biotic assimilation was responsible for the nutrient removal in period II, the difference in nutrient removal capability is directly related to the significant difference in biomass concentration between the HRAP + S (482.50 ± 43.41 mg SS L^{-1}) and the HRAP (395.45 ± 19.03 mg SS L^{-1}).

In periods III and IV, the TPBR showed a striking decrease in both TPRE and TNRE, and in period IV the removal efficiencies were significantly lower than in the HRAP and HRAP + S. Such a large decrease of the nutrient removal capability in the TPBR was directly related to the strong inhibition of the growth observed in these periods due to the high temperatures (Fig. 1D). Therefore the reduction of the photosynthetic activity of *S. obliquus* (Fig. 2D) directly affected not only biomass growth but also the nutrient removal capability.

The HRAP and HRAP + S presented an improvement of both TNRE and TNRE (Fig. 5A and B) in period III, and this was significantly higher in the HRAP + S than in the HRAP. This difference was again associated with the higher biomass concentration reached in the HRAP + S (522 ± 9.83 mg SS L^{-1}) than in the HRAP (460 ± 5.64 mg SS L^{-1}). Finally, in period IV, the HRAP + S did not show a significant increase in the average biomass concentration or nutrient removal efficiency, compared with period III (15 L min^{-1}). The HRAP, on the other hand, presented increases of both TPRE and TNRE when period II (15 L min^{-1}) and period IV (20 L min^{-1}) are compared, while at 15 L min^{-1}, in period III, it achieved removal efficiencies similar to those of the HRAP + S.

3.3. Biomass composition

Table 2 shows the biomass composition of the TPBR, HRAP + S and HRAP in the four different periods of operation in CM. The results for the TPBR in period IV are not presented due to the strong inhibition experienced in this period (Fig. 1D).

Regarding the carbon content in the biomass (% dry weight), there was a trend toward increase as the flue gas increases. In the three PBRs the minimum was reached in period I, when no flue gas was added. In this period I, significant differences were also observed between the PBRs. The carbon content reached in the TPBR (45.0 ± 2.2% C), was the highest while no significant

Table 2
Carbon, nitrogen and lipid content (% dry weight) of *Scenedesmus obliquus* in the high rate algal pond (HRAP), high rate algal pond with carbonation sump station (HRAP + S) and in the tubular airlift photobioreactor (TPBR) in the four periods of continuous mode of operation (periods I, II, III and IV); relationships between the inlet nutrient flow rates (mg TN or L^{-1} d^{-1} TP) and the concentration of microalgae in the reactor (g SS L^{-1}).

	TPBR			HRAP + S				HRAP			
	P I	P II	P III	P I	P II	P III	P IV	P I	P II	P III	P IV
C (% DW)	45.0 ± 2.2	46.7 ± 1.7	47.5 ± 2.0	40.2 ± 1.5	43.5 ± 1.8	45.0 ± 2.2	45.2 ± 1.7	40.0 ± 1.0	42.5 ± 1.6	45.0 ± 1.7	45.7 ± 2.0
N (% DW)	4.2 ± 0.2	3.9 ± 0.3	3.9 ± 0.3	4.0 ± 0.2	3.7 ± 0.2	3.2 ± 0.2	3.2 ± 0.1	4.2 ± 0.2	4.0 ± 0.2	3.6 ± 0.1	3.4 ± 0.1
Lipids (% DW)	22.0 ± 0.7	23.5 ± 0.5	23.8 ± 0.8	19.7 ± 0.7	24.3 ± 0.8	25.2 ± 0.9	25.7 ± 0.9	20.2 ± 0.5	23.4 ± 0.7	25.0 ± 0.6	25.5 ± 0.7
P:M (mg TP g SS^{-1} d^{-1})	0.85	0.68	0.75	1.01	0.54	0.54	0.47	0.91	0.65	0.61	0.49
N:M (mg TN g SS^{-1} d^{-1})	10.56	8.13	8.50	12.44	6.39	6.06	5.46	11.26	7.80	6.90	5.70

differences were observed between the HRAP + S (40.2 ± 1.5% C) and HRAP (40.0 ± 1.0% C). This higher carbon content in the TPBR could be attributed to the additional CO_2 in the air of the airlift pump system of the TPBR, which effectively reduces the carbon limitation in comparison with the HRAP and HRAP + S (Arbib et al., 2013). These differences in carbon content between different cultivation conditions have already been reported by Ruiz-Marin et al. (2010).

Once the flue gas was added (in periods II–IV), the carbon content showed an increasing trend in all the PBRs, with the lowest increase being observed in the TPBR; this was due mainly, as commented above, to the lower carbon limitation of the TPBR, and the differences between periods in this reactor were not significant. However, the HRAP and HRAP + S showed a significant difference in the carbon content between periods I and II, and as the flue gas flow rate was increased (in periods III and IV) there was also an increasing trend, but the differences in these periods were not significant. Under flue gas addition, the mean average carbon content percentages in the HRAP and HRAP + S were 44.4 ± 1.64 and 44.68 ± 0.98% C, respectively. These values are in accordance with data obtained by Molinuevo-Salces et al. (2010) for open cultivation systems.

Considering the changes in the nitrogen and lipid content in biomass, as the addition of flue gas was increased the biomass of all three PBRs shows a decrease of the nitrogen content and an increase of the lipid content. The decrease of the nitrogen content when CO_2 is added has been reported previously by several authors (Turpin, 1991; Faria et al., 2012). The simultaneous change of nitrogen and lipid content observed could be related to the N/M ratio (Table 2). The N/M ratio is the relationship between the input nutrient flow rate (mg TN or TP L^{-1} d^{-1}) and the concentration of microalgae in the reactor (g SS L^{-1}). Thus, as the flue gas was added, the biomass concentration increased (Fig. 2C and D) and at a constant concentration of nutrients in the influent wastewater (Table 1) both TP:M and TN:M decreased. Lower TN:M and TP:M ratios imply lower nutrient (nitrogen and phosphorus) availability for the microalgae, which promotes the production of biomass with less nitrogen reserves, and as a consequence of that, the biomass has a higher lipid content because of the stress (Guschina and Harwood, 2006; Pruvost et al., 2011). Both TN:M and TP:M in the HRAP and HRAP + S were lower than in the TPBR, except for the first period, when no flue gas was added; for the HRAP + S, TN:M decreased from 12.45 mg TN g SS^{-1} d^{-1} in period I to 5.45 mg TN g SS^{-1} d^{-1} in period IV, and consequently nitrogen content decreased from 4 ± 0.2 to 3.2 ± 0.15% N, while lipid content increased from 19.75 ± 0.75 to 25.75%.

4. Conclusions

Biomass productivity and nutrient removal efficiency were significantly improved when flue gas was added in the three PBRs, although this effect was lower in the TPBR due to the low carbon limitation. Flue gas added to the HRAP + S was more efficiently assimilated than in the HRAP, since the HRAP + S reached its maximum TNRE, TPRE and biomass productivity (92.15 ± 1.45%, 95.10 ± 0.84% and 19.77 ± 0.38 g m^{-2} d^{-1}, respectively) at a gas flow rate of 15 L min^{-1} whereas the HRAP presented similar results at a rate of 20 L min^{-1}. The carbonation sump station improved the flue gas mass transfer. Flue gas addition also promotes the production of biomass with less nitrogen reserves and, consequently, with a higher lipid content because of the stress due to nutrient limitation.

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